siRNA Mediated Inhibition of Gene Expression

PROTOCOL

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NOTE: This protocol is recommended for a well from a 6 well tissue culture plate. Adjust cell and reagent amounts proportionately for wells or dishes of different sizes.

- In a six well tissue culture plate, seed 2 x 10^5 cells per well in 2 ml antibiotic-free normal growth medium supplemented with FBS.
- Incubate the cells at 37° C in a CO_2 incubator until the cells are 60–80% confluent. This will usually take 18-24 hours.

NOTE: Healthy and subconfluent cells are required for successful transfection experiments. It is recommended to ensure cell viability one day prior to transfection.

- Prepare the following solutions:
  - **Solution A**: For each transfection, dilute 2–8 µl of siRNA duplex (i.e., 0.25–1 µg or 20–80 pmols siRNA) into 100 µl **siRNA Transfection Medium** (sc-36868).
  - **Solution B**: For each transfection, dilute 2–8 µl of **siRNA Transfection Reagent** (sc-29528) into 100 µl **siRNA Transfection Medium** (sc-36868). Peak activity should be at about 6 µl siRNA Transfection Reagent.

NOTE: Do not add serum and antibiotics to the **siRNA Transfection Medium** (sc-36868).

NOTE: Optimal siRNA amount used for transfection may vary for each target protein and should be determined experimentally.

NOTE: If a lower siRNA concentration is desired, dilute siRNA appropriately with **siRNA Dilution Buffer** (sc-29527).

NOTE: Although highly efficient in a variety of cell lines, **siRNA Transfection Reagent** (sc-29528) may not be suitable for use with all cell lines.

- Add the siRNA duplex solution (Solution A) directly to the dilute Transfection Reagent (Solution B) using a pipette. Mix gently by pipetting the solution up and down and incubate the mixture 15–45 minutes at room temperature.
- Wash the cells once with 2 ml of **siRNA Transfection Medium** (sc-36868). Aspirate the medium and proceed immediately to the next step.
- For each transfection, add 0.8 ml siRNA Transfection Medium to each tube containing the siRNA Transfection Reagent mixture (Solution A + Solution B). Mix gently and overlay the mixture onto the washed cells.
- Incubate the cells 5–7 hours at 37° C in a CO_2 incubator.

NOTE: Longer transfection times may be desirable depending on the cell line. However prolonged serum starvation may result in unwanted cell detachment or death.

NOTE: Fluorescein Conjugated Control siRNA should only be incubated for a total 5–7 hours at 37° C in a CO_2 incubator. At the end of incubation they are ready to be assayed by fluorescent microscopy.

- Add 1 ml of normal growth medium containing 2 times the normal serum and antibiotics concentration (2x normal growth medium) without removing the transfection mixture. If toxicity is a problem, remove the transfection mixture and replace with 1x normal growth medium.
- Incubate the cells for an additional 18–24 hours.
- Aspirate the medium and replace with fresh 1x normal growth medium.
- Assay the cells using the appropriate protocol 24–72 hours after the addition of fresh medium in the step above.

NOTE: Controls should always be included in siRNA experiments. Use either **Control siRNAs**: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 or sc-44238 or **Control siRNA (Fluorescein Conjugates)**: sc-36869, sc-44239, sc-44240 or sc-44241. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

NOTE: For Western blot analysis prepare cell lysate as follows: Wash cells once with PBS. Lyse cells in 300 µl 1x electrophoresis sample buffer (**Electrophoresis Sample Buffer, 2X**: sc-24945) by gently rocking the 6 well plate or by pipetting up and down. Sonicate the lysate on ice if necessary.

NOTE: For RT-PCR analysis isolate RNA using the method described by Chomczynski and Sacchi (Anal Biochem. 1987 Apr; 162(1): 156–159. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Chomczynski P, Sacchi N.) or a commercially available RNA isolation kit.